

Strategies for Managing Reproduction in the Heat-Stressed Dairy Cow¹

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ABSTRACT: Establishment and maintenance of pregnancy is difficult in lactating dairy cows exposed to heat stress because of reductions in estrous detection rate and the proportion of inseminated cows that maintain pregnancy. The most common approach to ameliorate heat stress in developed countries has been to alter the cow's environment through provision of shade, fans, sprinklers, and so on. Nonetheless, seasonal variation in reproductive function persists. Increased understanding of bovine reproductive function and its alteration by heat stress has led to additional strategies for reducing deleterious consequences of heat stress on reproduction. These include hormonally induced timed artificial insemination, which can reduce losses in reproductive efficiency caused by poor detection of estrus, and embryo transfer, which can increase pregnancy rate by allowing embryos to bypass the period when they are

most sensitive to elevated temperature (i.e., in the first 1 to 2 d after breeding). Other efforts are directed toward developing methods to protect the embryo from harmful actions of elevated temperature. Approaches being studied include manipulation of embryonic synthesis of heat shock proteins and use of antioxidants to reduce free radical damage associated with heat stress. It may also be possible to reduce the magnitude of hyperthermia caused by heat stress. This might be possible physiologically, for example by feeding of agents that affect thermoregulatory systems, or genetically by selecting for specific traits conferring thermal resistance. Finally, the development of bovine somatotropin as a lactational promont means that it may be possible to extend lactations beyond 305 d and voluntarily discontinue inseminations during periods of heat stress.

Key Words: Heat Stress, Embryo, Estrus, Fertility, Cattle

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Introduction: The Changing Nature of the Challenge of Heat Stress

Heat stress can be defined as the sum of forces external to a homeothermic animal that acts to displace body temperature from the resting state (Yousef, 1984). Such a stress can disrupt the physiol-

ogy and productive performance of an animal. The increase in body temperature caused by heat stress has direct, adverse consequences on cellular function. For instance, elevated temperature reduces the proportion of embryos that can continue in development (Ealy et al., 1995; Edwards and Hansen, 1997). Also, the physiological adaptations that homeotherms undergo during heat stress can compromise other physiologically important systems. An example is the redistribution of blood flow from the viscera to the periphery during heat stress. Although this adaptive response increases the dissipation of body heat to the environment, it also leads to reduced perfusion of the placental vascular bed (Alexander et al., 1987) and retarded fetal growth (Collier et al., 1982).

The impact of environmental temperatures on animal function have been alluded to since antiquity. In *On Airs, Waters and Places*, Hippocrates in the 5th

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century B.C. stated the belief that cattle raised in the Near East were more prolific than European cattle because of the mild climate. The first modern reports of summer depression in fertility were made over 50 years ago (Erb et al., 1940; Seath and Staples, 1941). Several trends will make the dilemma of heat stress even greater in the future than at present. Heat stress itself may increase in magnitude if continued global warming occurs (Hulme, 1997). Secondly, the world's population is growing faster in tropical and subtropical regions of the world than in temperate regions (Roush, 1994), and it is reasonable to assume that a greater proportion of the world's food animals will live in hot climates than is the case at present. Finally, changes in the genetics and physiology of food animals for increased production are making these animals less able to regulate body temperature, i.e., less adapted to warm environments. This is especially true for dairy cattle. Selection for milk yield reduces thermoregulatory ability in the face of heat stress (Berman et al., 1985) and magnifies the seasonal depression in fertility caused by heat stress (Al-Katanani et al., 1998). Also, use of bovine somatotropin (**BST**) as a lactational promotant can increase body temperatures during heat stress (West et al., 1991; Elvinger et al., 1992; Cole and Hansen, 1993). Moreover, adaptation of both beef and dairy cattle is reduced when native, genetically adapted cattle in tropical or semitropical regions are replaced by higher producing, unadapted breeds.

To Change the Environment or Change the Cow?

Two distinct and somewhat antithetical strategies have been implemented to increase production of dairy cattle in hot climates. One of these is to use breeds of cattle that are genetically adapted to the local environment. The second strategy is to alter the environment to reduce the magnitude of heat stress and allow cows to produce at their maximum genetic potential.

Use of genetically adapted breeds or crosses between European and local breeds is useful in areas of the world where high-quality feeds are scarce, where the price of milk is low, or where other environmental or economic factors make utilization of European breeds of cattle impractical. In studies in Brazil (Madelena et al., 1990) and Sudan (McGlothen et al., 1995), the genotype producing greatest amounts of milk depended on the level of management or feed input. When the level of management or feed was high, cattle with a preponderance of *Bos taurus* genetic background were favored whereas some more

intermediate type between *Bos indicus* and *Bos taurus* was superior when management or feed was low. One limitation to the use of crossbred cattle for dairy production is the high cost associated with maintaining purebred populations. Such a cost can be reduced through the use of in vitro production of embryos (Rutledge, 1997).

For dairy cattle in countries like the United States and Israel, where high level of milk yield is paramount to overall efficiency, the disparity in genetic ability for milk yield between European dairy breeds and other cattle has precluded use of tropically adapted genotypes in production systems. Similarly, selection for heat resistance within European breeds is made difficult by the inverse relationship between milk yield and body temperature regulation (Berman et al., 1985) and by the fact that dairy sires are not typically tested for genotype \times environment effects. Instead, the major approach to reduce effects of heat stress has been to ameliorate heat stress itself by modifying the environment.

The range of modifications to the environment used to cool dairy cattle has been reviewed elsewhere (Bucklin et al., 1991) as have results regarding use of environmental modification to improve reproductive performance (Hansen, 1997a). These modifications work to some extent and can reduce the negative effect of heat stress on milk yield and reproductive performance. They do not eliminate problems of heat stress, however, as illustrated by data in Figure 1, depicting seasonal variation in reproductive function of dairy cattle on a commercial dairy in Florida.

The fact that genetically adapted breeds are not practical in much of the world and that environmental modification has not eliminated the physiological effects of heat stress means that there is a need for additional methods to enhance physiological and productive functions during heat stress. This review will delineate some of these additional strategies with reference to reproductive function. The focus will be on improvement of herd pregnancy rate, which is defined as the product of insemination rate (proportion of eligible cows inseminated; except for timed breeding protocols, equivalent to estrus detection rate) and pregnancy rate per insemination. The term *herd pregnancy rate* is offered as an improvement over the term *pregnancy rate*, which is sometimes used with the same meaning as herd pregnancy rate but at other times is used to indicate pregnancy rate per insemination. Similarly, use of the term *conception rate* is avoided because, as usually used in the literature, it is a misnomer (few studies determine the actual fertilization rate but rather only the proportion of cows pregnant at some time after breeding). Heat stress

also affects other aspects of reproductive function including fetal growth and placental function during late pregnancy and fertility of bulls (see Hansen, 1997a for review). However, the situation during late pregnancy is deserving of separate treatment and AI can be used to bypass effects on the bull.

Improving herd pregnancy rate during heat stress involves reducing problems of estrous detection and embryonic mortality caused by heat stress. The proposed techniques are heavily dependent on regulating the physiology of the cow or embryo, either genetically or pharmacologically, to reduce or bypass the impact of heat stress on reproductive function.

Influence of Heat Stress on the Expression and Detection of Estrus

Changes in Estrus Caused by Heat Stress

Heat stress reduces the length (Monty and Wolff, 1974; Abilay et al., 1975) and intensity (Gangwar et al., 1965) of estrus. In Virginia, Nebel et al. (1997) reported that Holsteins in estrus during summer had 4.5 mounts per estrus vs 8.6 for those in winter. Changes in estrous activity caused by heat stress reduce the likelihood that estrus will be detected by dairy personnel. The percentage of undetected estrous periods on a commercial dairy in Florida were estimated at 76 to 82% during June through September vs 44 to 65% during October through May (Thatcher and Collier, 1986).

Some effects of heat stress may involve ACTH. Heat stress can cause increased cortisol secretion (Roman-Ponce et al., 1981; Wise et al., 1988a; Elvinger et al., 1992), and ACTH has been reported to block estradiol-induced sexual behavior (Hein and Allrich, 1992). In certain experiments, though, heat-stress induced elevations in circulating cortisol concentrations were transitory (Christison and Johnson, 1972; Miller and Alliston, 1974; Elvinger et al., 1992). In others, there was no increase in cortisol concentrations associated with heat stress (Wise et al., 1988b; West et al., 1991) or heat stress depressed cortisol concentrations (Abilay et al., 1975). Accordingly, effects of heat stress on estrous behavior likely include actions independent of the pituitary-adrenal axis. Some reports indicate that heat stress causes a reduction in peripheral concentrations of estradiol-17 β at estrus (Gwazdauskas et al., 1981; Wilson et al., 1998a) although this effect has not always been observed (Rosenberg et al., 1982). It is possible that the major reason that heat stress reduces the expression of estrus is because of the physical lethargy produced by

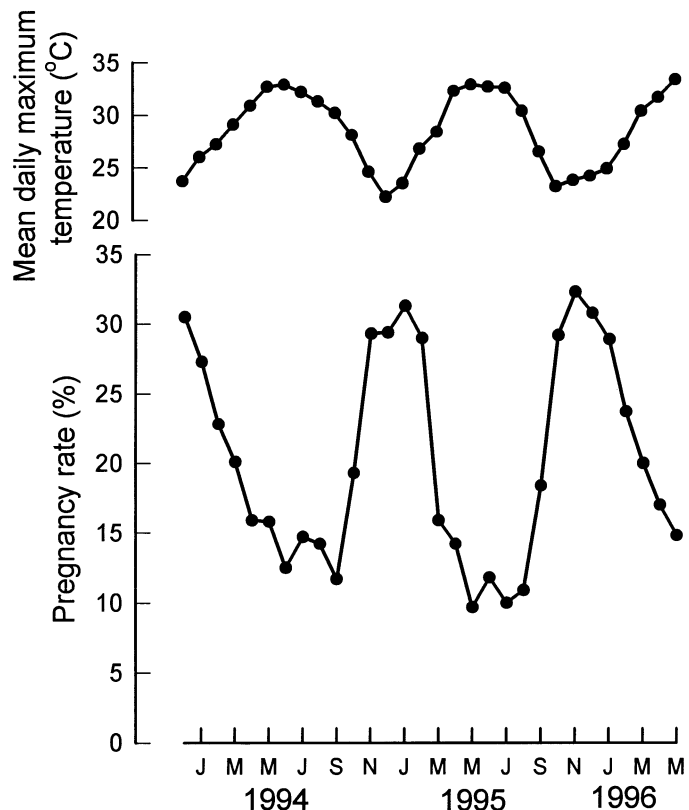


Figure 1. Seasonal variation in pregnancy rate (percentage of inseminations in which pregnancy was established) on a commercial dairy in South Florida in which cows were maintained in a facility with shade, fans, and sprinklers.

heat stress. Reduced physical activity is itself probably an adaptive response that limits heat production.

Aids for Detection of Estrus

Given the reduction in symptoms of estrus, one logical scheme to improve detection of estrus during hot weather is to make use of estrus detection aids. Several such systems have been developed, including application of paint to the tailhead (Macmillan et al., 1988), the HeatWatch system, in which a radiotelemetric pressure transducer is placed on the tailhead to transmit information regarding number of times an animal is mounted (Walker et al., 1996), and pedometers to measure increased locomotor activity associated with estrus (Maatje et al., 1997). There are little data as to whether these devices can improve estrus detection during heat stress. In a study conducted during the summer in Florida, the proportion of cows detected in estrus within 96 h following

Table 1. Effectiveness of timed insemination protocols for increasing pregnancy rates of lactating Holsteins when implemented during periods of heat stress in Florida^a

Exp. and treatment ^b	n	Interval from calving to first service (d)	Pregnancy rates		
			At first service	At d 90 postpartum	At d 120 postpartum
1					
BOE	184	82.4 ± 1.0	12.5 ± 2.5	9.8 ± 2.5	30.4 ± 3.5
TAI	169	72.4 ± 1.0***	13.6 ± 2.6	16.6 ± 2.6*	32.7 ± 3.6
2					
BOE	35	58.1 ± 1.7	8.6 ± 5.1	14.3 ± 7.2	37.1 ± 8.3
TAI	35	51.7 ± 1.7*	11.4 ± 5.1	34.3 ± 7.1 [†]	62.9 ± 8.3*
3					
PGF	156	91.0 ± 1.9	4.8 ± 2.5		16.5 ± 3.5
TAI	148	58.7 ± 2.1*	13.9 ± 2.6*		27.0 ± 3.6*

^aData represent least-squares means ± SEM.

^bExperiments 1 and 2: Aréchiga et al., 1998a; Experiment 3: de la Sota et al., 1998. BOE = breeding at each observed estrus beginning at d 70 (Experiment 1) or d 50 (Experiment 2) postpartum; TAI = timed artificial insemination programmed for d 70 (Experiment 1), 50 (Experiment 2) or d 60 (Experiment 3) followed by breeding at all observed estrous periods thereafter; PGF = injection of PGF at d 57 postpartum and breeding at all detected estrous periods thereafter.

[†] $P < .10$ ($P = .055$).

* $P < .05$.

** $P < .01$.

*** $P < .001$.

injection of prostaglandin $F_{2\alpha}$ (**PGF_{2α}**) was increased by the use of tail chalk from 24% to 43% (Ealy et al., 1994).

Natural Breeding

Some dairy producers make increased use of natural breeding during the summer to avoid the need for estrus detection. Indeed, the percentage of breedings performed using artificial insemination across the entire United States declines slightly in the summer (Powell and Norman, 1990). The genetic merit of sires used in the summer is also slightly lower than in the winter, at least in the United States (Powell and Norman, 1990). This decline is likely the result of greater use of lower-priced semen and natural breeding during hot weather.

The major liability of using natural breeding in hot weather is probably not the loss of genetic progress even though cows sired by natural service bulls produce income of \$150 to 200 per lactation less than cows sired by AI bulls (Funk, 1994). Given the current low rate of fertility in the summer in hot regions of the world, few animals will become pregnant following AI or natural breeding. Rather, the major difficulty occasioned by natural breeding is the deterioration of bull fertility caused by heat stress (see Hansen, 1997a for review). In fact, AI with frozen-thawed semen represents the best method for bypassing effects of heat stress on male fertility, and use of natural breeding squanders this benefit of AI.

Timed Artificial Insemination

The technology for manipulating follicular growth and ovulation has progressed far enough that good pregnancy rates can be achieved by insemination at a fixed time following programmed injections of GnRH or GnRH agonist and PGF_{2α} (Pursley et al., 1995; Burke et al., 1996; Pursley et al., 1997). Such timed artificial insemination (**TAI**) protocols eliminate the need for estrus detection. Implementation of a TAI protocol for first insemination after calving during periods of heat stress increased herd pregnancy rate (Table 1). This effect resulted primarily because interval from calving to first breeding was reduced. In the study of de la Sota et al. (1998), pregnancy rate to first service was also greater for cows bred by TAI as compared with control cows, perhaps because of poor diagnosis of estrus in the control group or an improvement in fertility in the TAI group caused by recruitment of a fresh dominant follicle. In contrast, there was no improvement in pregnancy rate to first service caused by TAI in the experiments of Aréchiga et al. (1998a).

Heat Stress-Induced Embryonic Mortality: Characteristics and Causes

Use of TAI protocols can eliminate problems of estrus detection caused by heat stress but is not sufficient to restore herd pregnancy rates to a level

seen in cool weather because of the severe consequences of heat stress for embryogenesis. Depression in pregnancy rate per insemination in warm or hot periods of the year has been well documented in many regions (Stott et al., 1962; Monty and Wolff, 1974; Rosenberg et al., 1977; Badinga et al., 1985; Cavestany et al., 1985; King et al., 1988; Du Preez et al., 1991; Ryan et al., 1993). There are two types of evidence that this depression is caused primarily by heat stress and not other environmental variables. Specifically, experimental application of heat stress can reduce fertility and embryonic survival (Dunlap and Vincent, 1971; Putney et al., 1988a, 1989b; Ealy et al., 1993), and cooling cows during the summer can improve pregnancy rate per insemination (Stott et al., 1972; Thatcher et al., 1974; Roman-Ponce et al., 1977; Wolfenson et al., 1988).

The mechanism by which heat stress causes decreased fertility is probably multifactorial and may vary depending on the magnitude of heat stress. When rectal temperatures in the hot season were low (39.0°C) because of environmental cooling, most early embryonic mortality associated with the hot season occurred between d 6 and 14 of pregnancy (Ryan et al., 1993). In contrast, Putney et al. (1988a) observed that experimental application of a heat stress that caused rectal temperatures to rise to 41.1°C caused a large reduction in embryonic development at an earlier time (d 7 after estrus). Since the process by which heat stress leads to embryonic loss may be different following mild vs severe heat stress, it follows that the optimal strategy for improving fertility during heat stress may depend on climatic conditions. Elucidation of the physiological and biochemical pathways through which heat stress causes disruption of embryonic survival are therefore important for development of techniques to intercept those pathways and to improve fertility.

Embryonic Development During Cleavage Stages

The periovulatory period and first few days of early pregnancy are very susceptible to disruption by heat stress. Exposure of superovulated heifers to heat stress for 10 h beginning at the onset of estrus had no effect on fertilization rate but reduced the proportion of normal embryos recovered on d 7 after estrus (Putney et al., 1989b). The experiment was designed to avoid effects of heat stress on spermatozoa; heifers were not inseminated until body temperatures had returned to normal. Similarly, heat stress at d 1 (Ealy et al., 1993) or d 1 to 3 after breeding (Dunlap and Vincent, 1971) reduced embryonic survival. Heat stress of superovulated cows at d 3, 5, or 7 after estrus

did not affect embryonic development or survival at d 8 (Ealy et al., 1993). This result indicates that, as for other species (Dutt, 1964; Tompkins et al., 1967; Wolfenson and Blum, 1988), effects of heat stress on embryonic survival decrease as embryos proceed through development.

Disruption of early embryonic development results from actions on the embryo itself or on the oviductal or uterine environment in which the embryo resides. Alliston and Ulberg (1961) used a reciprocal embryo transfer scheme in ewes to show that both the embryo and reproductive tract are compromised by heat stress, with greater effects being exerted on the embryo itself. Exposure of cultured bovine embryos to heat shock (a term used herein to mean exposure of cultured cells to any elevation in temperature above normal body temperature) can compromise subsequent development (Ealy et al., 1995; Edwards and Hansen, 1997; Sugiyama, 1999). For the experiments of Sugiyama, the temperatures that embryos were exposed to varied in a diurnal manner to match variations of rectal temperatures of dairy cows in the summer in Queensland, Australia. Thus, the elevated temperature that an embryo is exposed to when its dam becomes hyperthermic could lead directly to embryonic death. In addition, heat shock of maturing oocytes in culture can lead to reduced protein synthesis, fertilization rate, and subsequent developmental competence (Lenz et al., 1983; Edwards and Hansen, 1996; Sugiyama, 1999). However, development to the blastocyst stage was less damaged by heat shock when applied to oocytes than when applied to two-cell embryos (Edwards and Hansen, 1997).

The fact that the deleterious effects of maternal heat stress decline as pregnancy proceeds (Ealy et al., 1993) may reflect acquisition of thermal resistance by the preimplantation embryo as it progresses from the zygote to blastocyst. Heat shock caused a greater reduction in the proportion of cultured two-cell embryos that developed to the blastocyst stage than heat shock of four- to eight-cell embryos; morulae were unaffected by heat shock (Edwards and Hansen, 1997). Thus, susceptibility of embryos to heat shock in vitro appears to parallel the situation in vivo. Caution must be exercised when interpreting these data because stage differences in resistance of mouse embryos to heat shock was not apparent for all developmental end points (Aréchiga and Hansen, 1998). In particular, two-cell embryos were more affected by heat shock than were four-cell embryos or morulae when the end point was development to blastocyst but not when the end point was development to the hatched blastocyst stage.

If, as apparent, bovine embryos become more resistant to heat shock as development proceeds, the mechanism responsible is not understood. Once, it was hypothesized that early embryos (<8- to 16-cell stage) would be more susceptible to heat shock because these embryos are transcriptionally quiescent and unable to produce protective molecules such as heat shock protein 70 (**HSP70**) in response to heat shock. It is now known that heat shock can induce synthesis of HSP70 as early as the two-cell stage (Edwards and Hansen, 1996). Heat-induced HSP70 synthesis is the result of new transcription (Chandolia et al., 1999).

Later Embryonic Development

Though embryos are most sensitive to elevated temperature early in development, heat stress at more advanced times can reduce development. For example, heat stress compromised embryonic development when applied from d 8 to 16 of pregnancy (Biggers et al., 1987). In addition, Ryan et al. (1993) observed differences in embryonic loss of superovulated cows between cool and hot periods of year at d 13 or 14 of pregnancy but not at d 6 or 7. Furthermore, exposure of bovine embryos flushed from superovulated heifers at d 6 or 7 of pregnancy to heat shock in culture reduced cell number (Sugiyama, 1999).

Uterine and Oviductal Function

These tissues may be compromised during heat stress for several reasons. First, heat stress leads to a redistribution of blood flow from the visceral organs to the periphery; the resultant decreased perfusion of nutrients and hormones could compromise endometrial and oviductal function. Roman-Ponce et al. (1978) observed that the increase in uterine blood flow caused by injection of estradiol-17 β was reduced in cows not exposed to shade in summer compared with those receiving shade. Secretion of the hormones regulating reproductive tract function may also be altered by heat stress. Recent experiments suggest that heat stress can cause an increase in peripheral concentrations of estradiol-17 β between d 1 and 4 of the estrous cycle (Wolfenson et al., 1995) and a reduction from d 4 through 8 (Wolfenson et al., 1995) and 11 through 21 of the cycle (Wilson et al., 1998ab). These results must be compared with earlier reports of no effect of heat stress (Roman-Ponce et al., 1981; Wise et al., 1988ab). Similarly, heat stress has been reported to increase (Abilay et al., 1975; Roman-Ponce et al., 1981; Trout et al., 1998), decrease (Rosenberg et al., 1982; Younas et al., 1993), or have no effect (Wise et al., 1988a; Wolfenson et al., 1995; Wilson et

al., 1998ab) on peripheral concentrations of progesterone during the luteal phase of the estrous cycle.

Some of the variation in hormonal responses to heat stress probably reflects the fact that ovarian steroid concentrations are dependent not only on rate of secretion from ovarian tissue but also on rate of vascular perfusion of the ovary, on possible adrenal release (at least for progesterone), on metabolism in the liver and other organs, and on the degree of hemodilution or hemoconcentration. The extent to which heat stress affects these other physiological characteristics could lead to variable changes in steroid hormone concentrations in peripheral blood. For example, heat stress can cause either dilution, concentration, or no effect on blood plasma volume (Richards, 1985; McGuire et al., 1989; Johnson et al., 1991; Elvinger et al., 1992), and the nature of effect of heat stress on blood volume will affect steroid hormone concentrations in blood.

Actions of steroid hormones on reproductive tract tissue could conceivably be reduced during heat stress as a result of increased synthesis of heat shock proteins. Heat shock can lead to increased synthesis of HSP70 and heat shock protein 90 in endometrium (Malayer et al., 1988; Malayer and Hansen, 1990). Both these proteins are part of the complex of proteins associating with the progesterone and estrogen receptor (Johnson et al., 1996; Nair et al., 1996; Sabbah et al., 1996). Increased synthesis of heat shock proteins might alter assembly, transport, or binding activities of steroid receptors. Indeed, heat shock blocked estradiol-induced transcription of the vitellogenin gene in *Xenopus* hepatocytes (Wolffe et al., 1984).

Direct actions of elevated maternal temperature on the function of reproductive tract tissues remains a possibility although exposure of cultured endometrial explants to 43°C caused relatively small changes in protein and DNA synthesis (Putney et al., 1988b; Malayer et al., 1988; Malayer and Hansen, 1990). However, heat shocks of 42 and 43°C increased output of prostaglandins by cultured endometrium collected at d 17 of the estrous cycle (Putney et al., 1988b; Malayer et al., 1990), and exposure of cultured d-17 conceptuses to 43°C reduced secretion of interferon- γ (Putney et al., 1988b). Furthermore, heat stress on d 17 of pregnancy increased uterine production of prostaglandin F_{2 α} in response to oxytocin (Wolfenson et al., 1993).

Heat Shock of Spermatozoa

Fertilization rate can be reduced by maternal heat stress (Gordon et al., 1987; Monty and Racowsky, 1987). This effect could involve effects on sperm as

well as the oocyte because sperm deposited into the reproductive tract of a hyperthermic female are potentially at risk from damage due to heat shock. However, several indices of spermatozoal function, such as motility and acrosomal integrity, were little affected by culture at 41 or 42°C (Monterroso et al., 1995). In the rabbit, embryos formed from fertilization of oocytes with heat-shocked spermatozoa had compromised development (Ulberg and Burfening, 1967; Burfening and Ulberg, 1968), but whether a similar phenomenon exists in cattle is unknown.

Impairment of Follicular Development

The follicle destined to ovulate emerges as an antral follicle about 40 d before ovulation (Lussier et al., 1987). Therefore, heat stress during the period of follicular growth has the potential to compromise the oocyte, either because of direct actions of elevated temperature on the oocyte or because of alterations in follicular function that would compromise oocyte quality.

Heat stress can alter follicular dynamics by reducing follicular dominance. Badinga et al. (1993) observed that heat stress beginning on the day of ovulation reduced the diameter and volume of the dominant follicle on d 8 of the estrous cycle. Heat stress from d 3 to 5 of the estrous cycle increased androstenedione and reduced estradiol-17 β concentrations in follicular fluid of the dominant follicle collected at d 7 (Wolfenson et al., 1997). In another experiment (Wolfenson et al., 1995), heat stress beginning at d 1 of the estrous cycle caused an increase in number of follicles >10 mm in diameter, earlier emergence of the dominant follicle of the second follicular wave, and tended to reduce plasma concentrations of inhibin.

Initiation of heat stress at d 11 of the estrous cycle caused more estrous cycles characterized by three follicular waves vs two follicular waves, reduced estradiol-17 β concentrations in blood and caused estrous cycle extension (Wilson et al., 1998ab). The increase in estrous cycle length was ascribed to reduced estrogenic support for the uterine luteolytic mechanism. In another study, however, there was no extension of estrous cycle length caused by initiation of heat stress at d 11 (Trout et al., 1998).

Changes in follicular function in response to heat stress could be the result of endocrine changes in LH secretion (Wise et al., 1988a; Gilad et al., 1993) or changes in metabolic hormones that affect ovarian function. Heat stress tended to reduce concentrations of somatotropin (Igono et al., 1987; McGuire et al., 1991) but did not affect IGF-1 concentrations

(McGuire et al., 1991). Elevated body temperature may also directly affect follicular function. Culture of thecal cells at 40.5°C reduced androstenedione production from cultured thecal cells but generally had no effect on estradiol-17 β production from cultured granulosa cells (Wolfenson et al., 1997). Likewise, heat shock reduced LH-stimulated progesterone secretion from rat luteal cells in a process that could be partially blocked by antisense oligonucleotide to HSP70 (Khanna et al., 1995).

It is not known whether effects of heat stress on follicular development are sufficient to alter subsequent fertility and, if so, to what extent altered follicular development contributes to the summer decline in fertility. Recently, an analysis of Dairy Herd Improvement Association data from South Georgia and North Florida revealed results consistent with the possibility for fertility-altering consequences of heat stress before breeding (Al-Katanani et al., 1998). A subset of data was created that included only cows exposed to relatively cool temperatures (average dry bulb temperatures of < 25°C) from d 9 before breeding until d 1 before breeding. For this group of cows, those experiencing average air temperatures >20°C on day -10 before breeding had lower 90-d nonreturn rate than those cows experiencing air temperatures < 20°C. Although these results suggest that preovulatory effects of heat stress may be important for subsequent fertility, the definitive experiment in which heat stress is experimentally applied at various times before breeding remains to be performed. In sheep, such an experiment revealed that heat stress on d 12 before breeding reduced fertilization rate and lambing rate (Dutt, 1964).

Use of Embryo Transfer to Bypass Embryonic Mortality Caused by Heat Stress

The fact that early embryos appear particularly sensitive to heat stress has at least two implications for methods to improve fertility during hot weather. First, selected cooling during a few days in early pregnancy, called *strategic cooling* by Hansen et al. (1992), can cause some improvement in pregnancy rate per insemination (see Hansen, 1997a for review). Secondly, embryo transfer can be used to improve summer fertility by bypassing the effects of heat stress on early embryonic development. Embryos transferred into recipients at d 7 after estrus have already passed through the most thermosensitive periods of development (Ealy et al., 1993; Edwards and Hansen, 1997), and it is reasonable to expect that pregnancy rate to

Table 2. Summary of pregnancy rates in lactating cows in Florida following artificial insemination or embryo transfer in the summer^a

Experiment ^b	Treatment ^c	n	Pregnancy rate (%) ^d
1	AI	524	13.5 ^e
	ET, SO, unfrozen embryo	113	29.2
2	AI	84	21.4 ^f
	ET, SO, frozen embryo	48	35.4
	ET, IVF, frozen embryo	48	18.8
3	TAI	129	4.3 ^g
	TET, IVF, unfrozen embryo	133	17.0
	TET, IVF, frozen embryo	142	7.1

^aTable is reproduced from Hansen (1997b).

^bExperiment 1: Putney et al., 1989a; Experiment 2: Drost et al., 1994; Experiment 3: Ambrose et al., 1997.

^cAbbreviations are as follows: AI = bred via artificial insemination following detection of estrus; ET, transfer of embryo at 7 d after estrus; SO = embryo produced by superovulation; IVF = embryo produced by in vitro fertilization; TAI = GnRH-d 0, PGF_{2α}-d 7, GnRH-d 9, AI-16 h after second GnRH; TET = GnRH-d 0, PGF_{2α}-d 7, GnRH-d 9, ET-d 17 (i.e., 7 d after predicted estrus). Embryos were either transferred without freezing (unfrozen) or after freeze-thawing (frozen).

^dDetermined at d 40 to 60 (Putney et al., 1989a), 42 (Drost et al., 1994), or 45 (Ambrose et al., 1997) of pregnancy.

^eAI vs ET, $P < .001$.

^fAI vs ET-SO and ET-IVF, $P > .10$; ET-SO vs ET-IVF, $P < .02$.

^gTET-fresh vs TET-frozen and TAI, $P < .01$.

embryo transfer during the summer would be higher than pregnancy rate to AI. This has been demonstrated in a series of experiments by Thatcher and Drost and their colleagues (Table 2). Furthermore, there was no temperature-dependent depression in pregnancy rate following embryo transfer in a commercial embryo transfer unit in the southern United States (Figure 2).

There are several obstacles to the routine use of embryo transfer in summer although none of these should be insurmountable. Recovery of transferable embryos from superovulated recipients is reduced by heat stress (Monty and Racowsky, 1987; Gordon et al., 1987; Putney et al., 1988a). This limitation can be bypassed by the use of frozen embryos collected at cool periods of year or in regions not susceptible to heat stress. A second obstacle, the high cost of embryo transfer, can be alleviated through the use of in vitro fertilization (IVF) of oocytes collected using slaughterhouse material. Rutledge (1997) has estimated that the transmitting ability for milk production for oocytes from culled cows is only -148 lb of milk. Unfortunately, the technology for freezing IVF-derived embryos has not progressed to the point at which acceptable pregnancy rates have been achieved under heat stress conditions. Although transfer of fresh embryos produced by superovulation or IVF increased

pregnancy rate in summer as compared with AI, such was not the case when frozen IVF-derived embryos were used (Table 2).

Poor detection of estrus during heat stress could limit the number of cows eligible for embryo transfer. Recently, it has been shown that use of the ovulation synchronization regimen developed for TAI (i.e., GnRH-PGF_{2α}-GnRH) has the potential for synchronizing ovulation in embryo transfer recipients (Ambrose et al., 1997). Pregnancy rate following timed embryo transfer (i.e., at d 8 after the last GnRH injection) was greater for cows receiving an unfrozen IVF-derived embryo as compared with cows that were subjected to TAI (Table 2). The synchronization rate was 76.2%, and development of more effective ovulation synchronization procedures should make timed embryo transfer more effective also. One factor affecting success rate was body condition score; pregnancy rate improved as body condition score increased.

Manipulation of Induced Thermotolerance Reactions to Protect Embryos

In culture, embryos can be made more resistant to a severe heat shock if they are first exposed to a milder

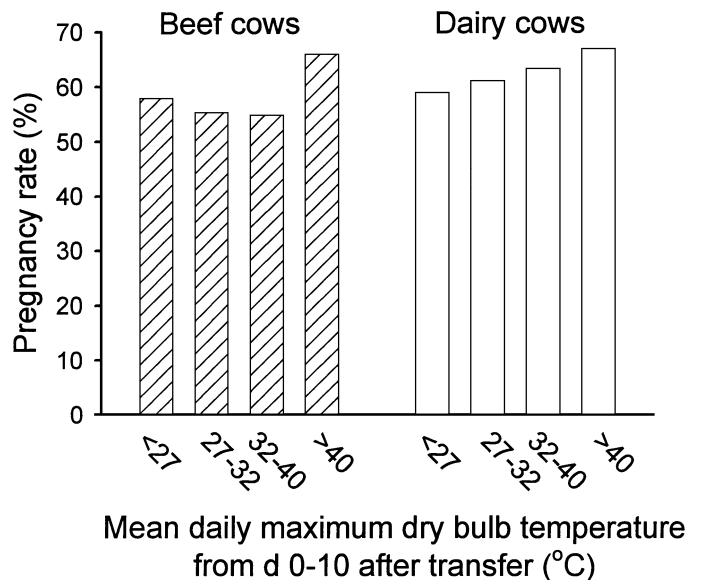


Figure 2. Pregnancy rates in lactating embryo transfer recipients as related to mean daily maximum dry bulb temperature during days 0-10 after transfer. Data, which are based on 18,453 transfers in beef cattle and 1,320 transfers in dairy cattle, are from Putney et al. (1988c).

heat shock. This phenomenon, termed *induced thermotolerance*, has been described for mouse eight-cell embryos, morulae, and blastocysts (Ealy and Hansen, 1994; Aréchiga et al., 1995; Aréchiga and Hansen, 1998) and for bovine blastocysts (Ealy and Hansen, 1994). The mechanism causing induced thermotolerance in embryos has not been elucidated but presumably involves induction of heat shock protein synthesis by the mild heat shock. In nonembryonic cells, heat shock proteins 90, 70, and 27 have been implicated in resistance of cells to heat shock (Johnston and Kucey, 1988; Riabowol et al., 1988; Landry et al., 1989; Mailhos et al., 1994) and injection of HSP70 mRNA into murine oocytes increased survival following exposure to 42°C for 1 h (Hendrey and Kola, 1991). If induced thermotolerance would protect embryos in vivo as well as in vitro, it might be possible to improve embryonic survival during hot weather by triggering the biochemical changes responsible for induced thermotolerance. To do so would be relatively easy for transferred embryos but would be more difficult for embryos produced by AI.

It is not known how early the biochemical mechanisms allowing induced thermotolerance develop. In mouse embryos that develop in utero, induced thermotolerance did not occur until embryos were morulae (Ealy et al., 1994). However, bovine embryos can produce increased amounts of HSP70 in response to heat shock as early as the two-cell stage (Edwards and Hansen, 1996; Edwards et al., 1997). One indirect piece of evidence for induced thermotolerance is the observation that exposure of bovine two-cell embryos to 40°C for 12 h did not compromise development even though development was reduced upon exposure to 40°C for 9 h (Rivera et al., 1998). Perhaps, the longer incubation was sufficient to induce some thermotolerance. In the same abstract, it was reported that attempts to make two-cell embryos resistant to a heat shock of 41°C for 12 h by prior exposure to 41 or 42°C for 80 min h were unsuccessful. Perhaps other temperature combinations may yield different results.

Antioxidants and Fertility

At least in culture, actions of heat shock on embryonic development involve increased production of free radicals. The evidence for this idea includes the fact that heat shock caused a reduction in the intracellular concentrations of the antioxidant glutathione in mouse morulae (Aréchiga et al., 1995) and that addition of various antioxidants to culture media, including taurine, glutathione, and vitamin E,

provided some thermoprotection to mouse (Malayer et al., 1992; Aréchiga et al., 1994, 1995) and cow morulae (Ealy et al., 1992). In vivo, exposure of cows to heat stress did not alter circulating concentrations of the antioxidants β -carotene or vitamin E or increase skeletal muscle content of malondialdehyde, a product of free radical oxidation of lipid membrane (Trout et al., 1998). In contrast, total antioxidant activity in blood plasma was reduced by heat stress for cows in environmental chambers and was negatively associated with temperature-humidity index among lactating cows (Harmon et al., 1997).

Several experiments have been conducted to test whether administration of antioxidants during heat stress increases fertility. Acute administration of vitamin E (given at breeding; Ealy et al., 1994) or β -carotene (given at d -6 to 0 before expected estrus; Aréchiga et al., 1998b) did not improve fertility of cows inseminated during warm periods of the year in Florida. Long-term (≥ 90 d) feeding of supplemental β -carotene did increase herd pregnancy rate for cows calving in Florida from May 2 to August 5 (Aréchiga et al., 1998a). The proportion of cows pregnant by d 120 postpartum was 21% in controls and 35% for cows fed supplemental β -carotene. Other studies regarding long-term administration of antioxidants are warranted before the benefits of such treatments are verified. One potential obstacle to development of antioxidant therapies is that early embryos—that is, those most sensitive to heat shock—may be refractory to the protective properties of antioxidants. In cattle, two-cell embryos were not protected from heat shock by addition of glutathione, glutathione ester, or taurine to culture medium (Ealy et al., 1995).

Regulation of Physiological Responses Controlling Body Temperature

Effects of heat stress on reproduction and other physiological functions are a direct consequence of the increase in body temperature caused by heat stress or of the physiological changes cows undergo to reduce the magnitude of hyperthermia. It follows, then, that one strategy for reducing adverse effects of heat stress on production is to modify thermoregulatory systems to reduce the body temperature rise coincident with heat stress. A great deal is known about the central nervous control of body temperature and the various pathways by which body heat is exchanged with the environment, but little work has been conducted to regulate these pathways to minimize hyperthermia during heat stress. There is some equivocal evidence

that feeding culture extracts of the fungus *Aspergillus oryzae* can decrease body temperatures of heat-stressed cows (Huber et al., 1994; Yu et al., 1997). The mechanism is unclear but could include actions on hypothalamic control centers for thermoregulation. Feeding supplemental niacin has also been reported to decrease effects of heat stress on milk yield and feed intake, perhaps by increasing heat loss through the cutaneous vasculature (Spain and Spiers, 1997).

Identification of Genes for Resistance to Heat Stress

Heritability estimates for rectal temperature under heat stress conditions range from .25 to .65 (Finch, 1986). Selection for low rectal temperature might lead to indirect selection for cows with low milk production because of the negative association between level of milk yield and resistance to heat stress (Berman et al., 1985). One option is to select for production under the constraints of the environment the animals are reared in. In one study (Frisch, 1981), selection for growth rate of cattle in a hot environment led to development of animals with increased thermal resistance.

Another alternative is to select for specific traits conferring resistance to heat stress. One trait that is easy to identify is hair pigment. In Holsteins, the proportion of the coat that is black is highly heritable (Becerril et al., 1994). Holstein cows that are predominately white have been reported to be less sensitive to heat stress than predominately black Holsteins with respect to changes in body temperature, milk yield, and reproduction (King et al., 1988; Hansen, 1990; Becerril et al., 1994) (Figure 3). This advantage has not always been seen, however (Godfrey and Hansen, 1996). Questions of skin damage from solar radiation must also be resolved. Recently, a gene controlling hair length has been described in the Senepol breed that can confer increased ability to regulate body temperature during heat stress (Olson et al., 1997). If hair length is important for thermoregulation in dairy cattle, it should prove feasible to introduce this gene into dairy breeds through conventional breeding practices.

Can genes be identified that confer cellular resistance to heat shock? If so, the frequency of these genes could be increased by genetic selection. Turner (1982) reported that the decrease in fertility caused by an increase in rectal temperature was of the same magnitude for *Bos taurus* and *Bos indicus* × *Bos taurus* cattle. This would suggest that the greater

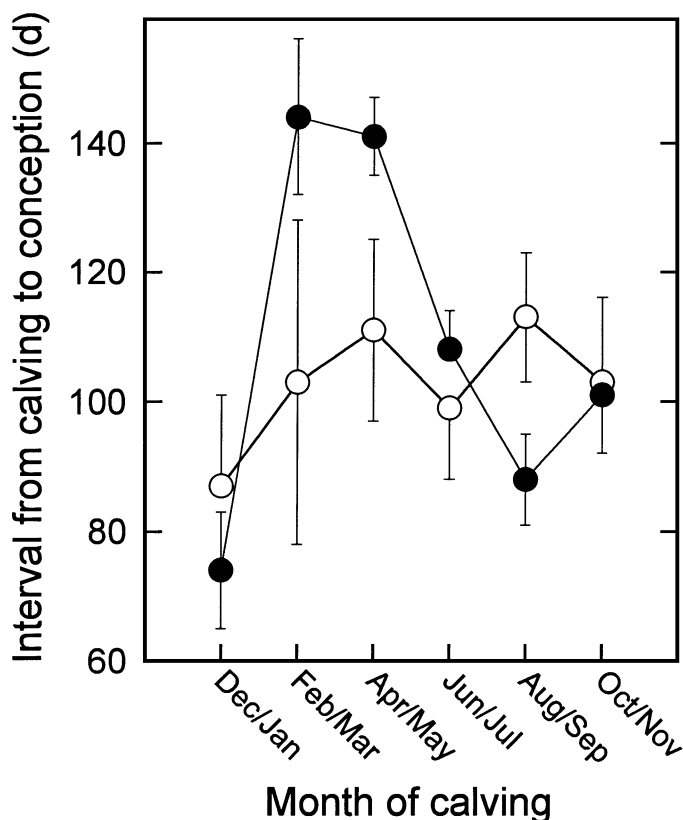


Figure 3. Seasonal variation in interval from calving to conception for Holstein cows that were predominately white (○) or black (●) in color. Cows were housed on a commercial dairy in Arizona. Data are from King et al. (1988).

resistance of the *Bos indicus* crosses to heat stress is the result of adaptations to regulate body temperature. Nonetheless, there have been reports that cells from breeds of cattle considered as thermally resistant respond different to temperature than cells from less thermally adapted breeds (Malayer and Hansen, 1990; Kamwanja et al., 1994). In the most striking example, survival of lymphocytes to a lethal heat shock was greater for cells from Brahman and Senepol cattle than for cells of Angus cattle (Kamwanja et al., 1994). These differences were not due to synthesis of HSP70 because the magnitude of heat induction of HSP70 was similar between breeds.

Scheduling Reproductive Activities to Avoid Heat Stress

One option is to avoid breeding cattle during hot times of year. Seasonal calving systems are practical

in some regions of the world but often are not because of economic losses associated with seasonal milk shortages. Development of BST may make seasonal calving more practical since BST can be used profitably to extend calving interval to 18 mo (Van Amburgh et al., 1997) and perhaps longer. Bovine somatotropin is effective in increasing milk yield under conditions of heat stress (Staples et al., 1988; Johnson et al., 1991; West et al., 1991; Elvinger et al., 1992). Nonetheless, heat abatement efforts should be used for BST-treated cows because of the potential for accentuated hyperthermia as compared with cows not treated with BST (West et al., 1991; Elvinger et al., 1992; Cole et al., 1993).

Conclusions and Implications

Designing dairy cattle housing to cool cows during hot periods of the year has reduced the magnitude of effects of heat stress on reproductive function but large declines in herd pregnancy rate still occur during periods of hot weather. Nonetheless, a combination of new reproductive technologies and innovative application of existing technologies offers prospects for achieving additional improvements in reproductive performance during heat stress.

Poor estrus detection. This problem can be solved through the use of TAI programs (Aréchiga et al., 1998a; de la Sota et al., 1998). Under this paradigm, high embryonic mortality following breeding will continue to limit herd pregnancy rates.

Increase in embryonic survival through embryo transfer. Pregnancy rates in summer can be improved through the use of embryo transfer (Putney et al., 1989a; Drost et al., 1994; Ambrose et al., 1997). For this technology to be widely adopted, further improvements must be made in the techniques for in vitro production of embryos, embryo freezing, and timed embryo transfer so as to lower costs and simplify animal handling.

Alteration of the biochemical properties of the embryo to protect it from exposure to elevated temperature. Cultured embryos can be made more thermotolerant to heat shock by exposure to a mild heat shock (Ealy and Hansen, 1994; Aréchiga et al., 1995; Aréchiga and Hansen, 1998). Additional challenges will be to identify the molecules responsible for this phenomenon of induced thermotolerance and develop strategies for achieving thermoprotection in vivo. Antioxidants have reduced effects of heat shock on cultured embryos (Malayer et al., 1992; Ealy et al., 1992; Aréchiga et al., 1994, 1995) and, in one experiment

(Aréchiga et al., 1998a), increased herd pregnancy rate in summer. Nonetheless, additional studies evaluating effectiveness of antioxidant administration are warranted before use of antioxidant supplementation can be recommended.

Genetic modification. Genetic selection has been a traditional method to reduce effects of environment on livestock by development of animals that are genetically adapted to hot climates. There are additional possibilities for meeting this goal. Identification of specific genes that control traits related to thermotolerance make it possible to select for thermal resistance without inadvertently selecting against milk yield. Examples of such traits include coat color (King et al., 1988; Hansen, 1990; Becerril et al., 1994) and genes controlling hair length (Olson et al., 1997). Possibly, there are also genes controlling cellular resistance to heat shock (Kamwanja et al., 1994). Integration of marker-assisted selection (Meuwissen and Van Arendonk, 1992; Dodgson et al., 1997; Kappes, 1999) into animal breeding systems should make selection for traits conferring thermotolerance more rapid. Crossbreeding schemes are likely to be important in certain dairy production systems (Madelena et al., 1990) and use of in vitro production of embryos makes maintenance of the purebred herds required to produce crossbreds less of a limitation to these breeding programs (Rutledge, 1997).

Pharmaceuticals and nutraceuticals. There are prospects for development of pharmaceuticals or feedstuffs (nutraceuticals) that regulate heat production and heat loss mechanisms; perhaps fungal culture extracts represent the first example of such preparations (Huber et al., 1994). In addition, an existing pharmaceutical, BST, can prove useful for extending lactation (Van Amburgh et al., 1997) and avoiding inseminations during hot periods of year.

Taken together then, it is evident that reproductive dysfunction caused by heat stress can be reduced by 1) reducing the magnitude of heat stress that drives cattle into hyperthermia, 2) changing the cow physiologically or genetically so that the degree of hyperthermia caused by heat stress is reduced, 3) manipulating the cow's reproductive system to bypass phases of the reproductive process that are particularly sensitive to heat stress or 4) use of lactational promotants like BST to avoid breeding cows during seasons of the year when heat stress can compromise reproduction. Although not all of these approaches will prove feasible under practical conditions, it should be manifest that there are myriad opportunities for solving what is the one of the most severe environmental limitations to cattle reproduction.

Literature Cited

- Abilay, T. A., H. D. Johnson, and M. Madan. 1975. Influence of environmental heat on peripheral plasma progesterone and cortisol during the bovine estrous cycle. *J. Anim. Sci.* 58: 1836–1840.
- Alexander, G., J.R.S. Hales, D. Stevens, and J. B. Donnelly. 1987. Effects of acute and prolonged exposure to heat on regional blood flows in pregnant sheep. *J. Dev. Physiol.* 9:1–15.
- Al-Katanani, Y. M., D. W. Webb, and P. J. Hansen. 1998. Factors affecting seasonal variation in non-return rate of lactating dairy cows. *J. Dairy Sci.* 81 (Suppl. 1):217 (Abstr.).
- Alliston, C. W., and L. C. Ulberg. 1961. Early pregnancy loss in sheep at ambient temperatures of 70° and 90°F as determined by embryo transfer. *J. Anim. Sci.* 20:608–613.
- Ambrose, J. D., M. Drost, R. L. Monson, J. J. Rutledge, M. L. Liebfried-Rutledge, M. J. Thatcher, T. Kassa, M. Binelli, P. J. Hansen, P. J. Chenoweth, and W. W. Thatcher. 1997. Timed embryo transfer in heat-stressed dairy cattle: A field trial with IVF-derived embryos. *J. Dairy Sci.* 80 (Suppl. 1):239 (Abstr.).
- Aréchiga, C. F., A. D. Ealy, and P. J. Hansen. 1994. Efficacy of vitamin E and glutathione for thermoprotection of murine morulae. *Theriogenology* 41:1545–1553.
- Aréchiga, C. F., A. D. Ealy, and P. J. Hansen. 1995. Evidence that glutathione is involved in thermotolerance of preimplantation mouse embryos. *Biol. Reprod.* 52:1296–1301.
- Aréchiga, C. F., and P. J. Hansen. 1998. Response of preimplantation murine embryos to heat shock as modified by developmental stage and glutathione status. *In Vitro Cell. Dev.-Anim.* 34: 655–659.
- Aréchiga, C. F., C. R. Staples, L. R. McDowell, and P. J. Hansen. 1998a. Effects of timed insemination and supplemental β -carotene on reproduction and milk yield of dairy cows under heat stress. *J. Dairy Sci.* 81:390–402.
- Aréchiga, C. F., S. Vázquez-Flores, O. Ortiz, J. Hernández-Cerón, A. Porras, L. R. McDowell, and P. J. Hansen. 1998b. Effect of injection of β -carotene or vitamin E and selenium on fertility of lactating dairy cows. *Theriogenology* 50:65–76.
- Badinga, L., W. W. Thatcher, T. Diaz, M. Drost, and D. Wolfenson. 1993. Effect of environmental heat stress on follicular development and steroidogenesis in lactating Holstein cows. *Theriogenology* 39:797–810.
- Becerril, C. M., C. J. Wilcox, M. S. Campos, and P. J. Hansen. 1994. Genetic effects and relationship of milk production and percentage of white coat in a subtropical Holstein herd. *Rev. Brasil. Genet.* 17:65–68.
- Berman, A., Y. Folman, M. Kaim, M. Mamen, Z. Herz, D. Wolfenson, A. Arieli, and Y. Graber. 1985. Upper critical temperatures and forced ventilation effects for high-yielding dairy cows in a subtropical environment. *J. Dairy Sci.* 68:1488–1495.
- Biggers, B. G., R. D. Geisert, R. P. Wetteman, and D. S. Buchanan. 1987. Effect of heat stress on early embryonic development in the beef cow. *J. Anim. Sci.* 64:1512–1518.
- Bucklin, R. A., L. W. Turner, D. K. Beede, D. R. Bray, and R. W. Hemken. 1991. Methods to relieve heat stress for dairy cows in hot climates. *Appl. Eng. Agric.* 7:241–247.
- Burfening, P. J., and L. C. Ulberg. 1968. Embryonic survival subsequent to culture of rabbit spermatozoa at 38° and 40°C. *J. Reprod. Fertil.* 15:87–92.
- Burke, J. M., R. L. de la Sota, C. A. Risco, C. R. Staples, E.J.P. Schmitt, and W. W. Thatcher. 1996. Evaluation of timed insemination using a gonadotropin-releasing hormone agonist in lactating dairy cows. *J. Dairy Sci.* 79:1385–1993.
- Cavestany D., A. B. El-Wishy, and R. H. Foote. 1985. Effect of season and high environmental temperature on fertility of Holstein cattle. *J. Dairy Sci.* 68:1471–1478.
- Chandolia, R. K., M. R. Peltier, W. Tian, and P. J. Hansen. 1999. Evidence for transcription in early bovine embryos. *Theriogenology* 51:183 (Abstr.).
- Christison, G. I., and H. D. Johnson. 1972. Cortisol turnover in heat-stressed cows. *J. Anim. Sci.* 35:1005–1011.
- Cole, J. A., and P. J. Hansen. 1993. Effects of administration of recombinant bovine somatotropin on the responses of lactating and nonlactating cows to heat stress. *J. Am. Vet. Med. Assn.* 203:113–117.
- Collier, R. J., S. G. Doelger, H. H. Head, W. W. Thatcher, and C. J. Wilcox. 1982. Effects of heat stress during pregnancy on maternal hormone concentrations, calf birth weight and postpartum milk yield of Holstein cows. *J. Anim. Sci.* 54:309–319.
- de la Sota, R. L., J. M. Burke, C. A. Risco, F. Moreira, M. A. DeLorenzo, and W. W. Thatcher. 1998. Evaluation of timed insemination during summer heat stress in lactating dairy cattle. *Theriogenology* 49:761–770.
- Dodgson, J. B., H. H. Cheng, and R. Okimoto. 1997. DNA marker technology: a revolution in animal genetics. *Poult. Sci.* 76: 1108–1114.
- Drost, M., M.-J. D. Thatcher, C. K. Cantrell, K. E. Wolfsdorf, J. F. Hasler, and W. W. Thatcher. 1994. Conception rates after artificial insemination or transfer of frozen/thawed embryos to lactating cows during summer. *J. Dairy Sci.* 77 (Suppl. 1):380 (Abstr.).
- Dunlap, S. K., and C. K. Vincent. 1971. Influence of postbreeding thermal stress on conception rate in beef cattle. *J. Anim. Sci.* 32:1216–1218.
- Du Preez, J. H., S. J. Terblanche, W. H. Giesecke, C. Maree, and M. C. Welding. 1991. Effect of heat stress on conception in a dairy herd model under South African conditions. *Theriogenology* 35: 1039–1049.
- Dutt, R. H. 1964. Detrimental effects of high ambient temperature on fertility and early embryo survival in sheep. *Int. J. Biometeorol.* 8:47–56.
- Ealy, A. D., C. F. Aréchiga, D. R. Bray, C. A. Risco, and P. J. Hansen. 1994. Effectiveness of short-term cooling and vitamin E for alleviation of infertility induced by heat stress in dairy cows. *J. Dairy Sci.* 77:3601–3607.
- Ealy, A. D., M. Drost, C. M. Barros, and P. J. Hansen. 1992. Thermoprotection of preimplantation bovine embryos from heat shock by glutathione and taurine. *Cell Biol. Int. Rep.* 16: 125–131.
- Ealy, A. D., M. Drost, and P. J. Hansen. 1993. Developmental changes in embryonic resistance to adverse effects of maternal heat stress in cows. *J. Dairy Sci.* 76:2899–2905.
- Ealy, A. D., and P. J. Hansen. 1994. Induced thermotolerance during early development of murine and bovine embryos. *J. Cell. Physiol.* 160:463–468.
- Ealy, A. D., J. L. Howell, V. H. Monterroso, C. F. Aréchiga, and P. J. Hansen. 1995. Developmental changes in sensitivity of bovine embryos to heat shock and use of antioxidants as thermoprotectants. *J. Anim. Sci.* 73:1401–1407.
- Edwards, J. L., A. D. Ealy, V. H. Monterroso, and P. J. Hansen. 1997. Ontogeny of temperature-regulated heat shock protein 70 synthesis in preimplantation bovine embryos. *Mol. Reprod. Dev.* 48:25–33.
- Edwards, J. L., and P. J. Hansen. 1996. Elevated temperature increases heat shock protein 70 synthesis in bovine two-cell embryos and compromises function of maturing oocytes. *Biol. Reprod.* 55:340–346.

- Edwards, J. L., and P. J. Hansen. 1997. Differential responses of bovine oocytes and preimplantation embryos to heat shock. *Mol. Reprod. Dev.* 46:138–145.
- Elvinger, F., R. P. Natzke, and P. J. Hansen. 1992. Interactions of heat stress and bovine somatotropin affecting physiology and immunology of lactating cows. *J. Dairy Sci.* 75:449–462.
- Erb, R. E., J. W. Wilbur, and J. H. Hilton. 1940. Some factors affecting breeding efficiency in dairy cattle. *J. Dairy Sci.* 23:549 (Abstr.).
- Finch, V. A. 1986. Body temperature in beef cattle: its control and relevance to production in the tropics. *J. Anim. Sci.* 62:531–542.
- Frisch, J. E. 1981. Changes occurring in cattle as a consequence of selection for growth rate in a stressful environment. *J. Agr. Sci. (Camb.)* 96:23–38.
- Funk, D. A. 1994. Breeding dairy cattle—deciding what genetics to use. *Proc. Natl. Reprod. Symp., Pittsburgh*, pp. 65–74.
- Gangwar, P. C., C. Branton, and D. L. Evans. 1965. Reproductive and physiological response of Holstein heifers to controlled and natural climatic conditions. *J. Dairy Sci.* 48:222–227.
- Gilad, E., R. Meidan, A. Berman, Y. Graber, and D. Wolfenson. 1993. Effect of tonic and GnRH-induced gonadotrophin secretion in relation to concentration of oestradiol in plasma of cyclic cows. *J. Reprod. Fertil.* 99:315–321.
- Godfrey, R. W., and P. J. Hansen. 1996. Reproduction and milk yield of Holstein cows in the US Virgin Islands as influenced by time of year and coat color. *Arch. Latinamer. Prod. Anim.* 4:31–44.
- Gordon, I., M. P. Boland, H. McGovern, and G. Lynn. 1987. Effect of season on superovulatory responses and embryo quality in Holstein cattle in Saudi Arabia. *Theriogenology* 27:231 (Abstr.).
- Gwazdauskas, F. C., W. W. Thatcher, C. A. Kiddy, M. J. Paape, and C. J. Wilcox. 1981. Hormonal patterns during heat stress following PGF_{2α}-tham salt induced luteal regression in heifers. *Theriogenology* 16:271–285.
- Hansen, P. J. 1990. Effects of coat colour on physiological and milk production responses to solar radiation in Holsteins. *Vet. Rec.* 127:333–334.
- Hansen, P. J. 1997a. Effects of environment on bovine reproduction. In: R.S. Youngquist (Ed.) *Current Therapy in Large Animal Theriogenology*. pp 403–415. W. B. Saunders, Philadelphia.
- Hansen, P. J. 1997b. Strategies for enhancing reproduction of lactating dairy cows exposed to heat stress. *Proc. 16th Ann. Convention Am. Embryo Transfer Assn., Madison*, pp 62–72.
- Hansen, P. J., W. W. Thatcher, and A. D. Ealy. 1992. Methods for reducing effects of heat stress on pregnancy. In: H.H. Van Horn and C.J. Wilcox (Ed.) *Large Dairy Herd Management*. pp 116–125. Am. Dairy Sci. Assn., Savoy, IL.
- Harmon, R. J., M. Lu, D. S. Trammell, B. A. Smith, J. N. Spain, and D. Spiers. 1997. Influence of heat stress and calving on antioxidant activity in bovine blood. *J. Dairy Sci.* 80 (Suppl. 1):264 (Abstr.).
- Hein, K. G., and R. D. Allrich. 1992. Influence of exogenous adrenocorticotrophic hormone on estrous behavior in cattle. *J. Anim. Sci.* 70:243–247.
- Hendrey, J., and I. Kola. 1991. Thermolability of mouse oocytes is due to the lack of expression and/or inducibility of Hsp70. *Mol. Reprod. Dev.* 28:1–8.
- Huber, J. T., G. Higginbotham, R. A. Gomez-Alarcon, R. B. Taylor, K. H. Chen, S. C. Chan, and Z. Wu. 1994. Heat stress interactions with protein, supplemental fat, and fungal cultures. *J. Dairy Sci.* 77:2080–2090.
- Hulme, M. 1997. Global warming. *Prog. Phys. Geogr.* 21:446–453.
- Igono, M. O., H. D. Johnson, B. J. Stevens, G. F. Krause, and M. D. Shanklin. 1987. Physiological, productive, and economic benefits of shade, spray, and fan system versus shade for Holstein cows during summer heat. *J. Dairy Sci.* 70:1069–1079.
- Johnson, H. D., R. Li, W. Manalu, and K. J. Spencer-Johnson. 1991. Effects of somatotropin on milk yield and physiological responses during summer farm and hot laboratory conditions. *J. Dairy Sci.* 74:1250–1262.
- Johnson, J., R. Corbisier, B. Stensgard, and D. Toft. 1996. The involvement of p23, hsp90, and immunophilins in the assembly of progesterone receptor complexes. *J. Steroid Biochem. Mol. Biol.* 56:31–37.
- Johnston, R. N., and B. L. Kucey. 1988. Competitive inhibition of hsp70 gene expression causes thermosensitivity. *Science* 242:1551–1554.
- Kamwanja, L. A., C. C. Chase, Jr., J. A. Gutierrez, V. Guerriero, Jr., T. A. Olson, A. C. Hammond, and P. J. Hansen. 1994. Responses of bovine lymphocytes to heat shock as modified by breed and antioxidant status. *J. Anim. Sci.* 74:438–444.
- Kappes, S. M. 1999. Utilization of gene mapping information in livestock animals. *Theriogenology* 51:135–148.
- Khanna, A., R. F. Aten, and H. R. Behrman. 1995. Heat shock protein-70 induction mediates luteal regression in the rat. *Mol. Endocrinol.* 9:1431–1440.
- King, V. L., S. K. Denise, D. V. Armstrong, M. Torabi, and F. Wiersma. 1988. Effects of a hot climate on the performance of first lactation Holstein cows grouped by coat color. *J. Dairy Sci.* 71:1093–1096.
- Landry, J., P. Chretien, H. Lambert, E. Hickey, and L. A. Weber. 1989. Heat shock resistance conferred by expression of the human HSP27 gene in rodent cells. *J. Cell Biol.* 109:7–15.
- Lenz, R. W., G. D. Ball, M. L. Liebfried, R. L. Ax, and N. L. First. 1983. In vitro maturation and fertilization of bovine oocytes are temperature-dependent processes. *Biol. Reprod.* 29:173–179.
- Lussier, J. G., P. Matton, and J. J. Dufour. 1987. Growth rates of follicles in the ovary of the cow. *J. Reprod. Fertil.* 81:301–307.
- Maatje, K., S. H. Loeffler, and B. Engel. 1997. Optimal time of insemination in cows that show visual signs of estrus by estimating onset of estrus with pedometers. *J. Dairy Sci.* 80:1098–1105.
- Macmillan, K. L., K. V. Kaufa, D. R. Barnes, A. M. Day, and R. Henry. 1988. Detecting estrus in synchronized heifers using tailpaint and an aerosol raddle. *Theriogenology* 30:1099–1114.
- Madalena, F. E., A. M. Lemos, R. L. Teodoro, R. T. Barbosa, and J.B.N. Monteiro. 1990. Dairy production and reproduction in Holstein-Friesian and Guzera crosses. *J. Dairy Sci.* 73:1872–1886.
- Mailhos, C., M. K. Howard, and D. S. Latchman. 1994. Heat shock proteins hsp90 and hsp70 protect neuronal cells from thermal stress but not from programmed cell death. *J. Neurochem.* 63:1787–1795.
- Malayer, J. R., and P. J. Hansen. 1990. Differences between Brahman and Holstein cows in heat-shock induced alterations of protein secretion by oviducts and uterine endometrium. *J. Anim. Sci.* 68:266–280.
- Malayer, J. R., P. J. Hansen, and W. C. Buhi. 1988. Effect of day of the oestrous cycle, side of the reproductive tract and heat shock on in vitro protein secretion by bovine endometrium. *J. Reprod. Fertil.* 84:567–578.
- Malayer, J. R., J. W. Pollard, and P. J. Hansen. 1992. Modulation of thermal killing of bovine lymphocytes and preimplantation mouse embryos by alanine and taurine. *Am. J. Vet. Res.* 53:689–694.
- McGlothen, M. E., F. El Amin, C. J. Wilcox, and R. H. Davis. 1995. Effects on milk yield of crossbreeding Zebu and European breeds in the Sudan. *Rev. Brasil. Genet.* 18:221–228.

- McGuire, M. A., D. K. Beede, M. A. DeLorenzo, C. J. Wilcox, G. B. Huntington, C. K. Reynolds, and R. J. Collier. 1989. Effects of thermal stress and level of feed intake on portal plasma flow and net fluxes of metabolites in lactating Holstein cows. *J. Anim. Sci.* 67:1050–1060.
- McGuire, M. A., D. K. Beede, R. J. Collier, F. C. Buonomo, M. A. DeLorenzo, C. J. Wilcox, G. B. Huntington, and C. K. Reynolds. 1991. Effects of acute thermal stress and amount of feed intake on concentrations of somatotropin, insulin-like growth factor (IGF)-I and IGF-II, and thyroid hormones in plasma of lactating Holstein cows. *J. Anim. Sci.* 69:2050–2056.
- Meuwissen, T. H., and J. A. Van Arendonk. 1992. Potential improvements in rate of genetic gain from marker-assisted selection in dairy cattle breeding schemes. *J. Dairy Sci.* 75:1651–1659.
- Miller, H. L., and C. W. Alliston. 1974. Plasma corticoids of Angus heifers in programmed circadian temperatures of 17 to 21 C and 21 to 34 C. *J. Anim. Sci.* 38:819–822.
- Monterroso, V. H., K. C. Drury, A. D. Ealy, J. L. Howell, and P. J. Hansen. 1995. Effect of heat shock on function of frozen/thawed bull spermatozoa. *Theriogenology* 44:947–961.
- Monty, D. E., and C. Racowsky. 1987. In vitro evaluation of early embryo viability and development in summer heat-stressed, superovulated dairy cows. *Theriogenology* 28:451–465.
- Monty, D. E., and L. K. Wolff. 1974. Summer heat stress and reduced fertility in Holstein-Friesian cows in Arizona. *Am. J. Vet. Res.* 35:1496–1500.
- Nair, S. C., E. J. Toran, R. A. Rimerman, S. Hjerstad, T. E. Smithgall, and D. F. Smith. 1996. A pathway of multi-chaperone interactions common to diverse regulatory proteins: estrogen receptor, Fes tyrosine kinase, heat shock transcription factor Hsf1, and the aryl hydrocarbon receptor. *Cell Stress Chaperones* 1:237–250.
- Nebel, R. L., S. M. Jobst, M.B.G. Dransfield, S. M. Pandolfi, and T. L. Bailey. 1997. Use of radio frequency data communication system, HeatWatch®, to describe behavioral estrus in dairy cattle. *J. Dairy Sci.* 80 (Suppl. 1):179 (Abstr.).
- Olson, T. A., A. C. Hammond, and C. C. Chase, Jr. 1997. Evidence for the existence of a major gene influencing hair length and heat tolerance in Senepol cattle. *J. Anim. Sci.* 75 (Suppl. 1):147 (Abstr.).
- Powell, R. L., and H. D. Norman. 1990. Impact of changes in genetic improvement programs and annual cycles on Holstein service sire merit. *J. Dairy Sci.* 73:1123–1129.
- Pursley, J. R., M. R. Kosorok, and M. C. Wiltbank. 1997. Reproductive management of lactating dairy cows using synchronization of ovulation. *J. Dairy Sci.* 80:301–306.
- Pursley, J. R., M. O. Mee, and M. C. Wiltbank. 1995. Synchronization of ovulation in dairy cows using PGF_{2α} and GnRH. *Theriogenology* 44:915–923.
- Putney, D. J., M. Drost, and W. W. Thatcher. 1988a. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperature between days 1 to 7 post insemination. *Theriogenology* 30:195–209.
- Putney, D. J., M. Drost, and W. W. Thatcher. 1989a. Influence of summer heat stress on pregnancy rates of lactating dairy cattle following embryo transfer or artificial insemination. *Theriogenology* 31:765–778.
- Putney, D. J., J. R. Malayer, T. S. Gross, W. W. Thatcher, P. J. Hansen, and M. Drost. 1988b. Heat-stress induced alterations in the synthesis and secretion of proteins and prostaglandins by cultured bovine conceptuses and uterine endometrium. *Biol. Reprod.* 39:717–728.
- Putney, D. J., S. Mullins, W. W. Thatcher, M. Drost, and T. S. Gross. 1989b. Embryonic development in superovulated dairy cattle exposed to elevated ambient temperatures between the onset of estrus and insemination. *Anim. Reprod. Sci.* 19:37–51.
- Putney, D. J., W. W. Thatcher, M. Drost, J. M. Wright, and M. A. DeLorenzo. 1988c. Influence of environmental temperature on reproductive performance of bovine embryo donors and recipients in the southwest region of the United States. *Theriogenology* 30:905–922.
- Riabowol, K. T., L. A. Mizzen, and W. J. Welch. 1988. Heat shock is lethal to fibroblasts microinjected with antibodies against hsp70. *Science* 242:433–436.
- Richards, J. I. 1985. Effect of high daytime temperature on the intake and utilization of water in lactating Friesian cows. *Trop. Anim. Health Prod.* 17:209–217.
- Rivera, R. M., Y. M. Al-Katanani, and P. J. Hansen. 1998. Response of 2-cell bovine embryos to heat shock: effect of magnitude of heat shock and possible induced thermotolerance. *J. Dairy Sci.* 81 (Suppl. 1):219 (Abstr.).
- Roman-Ponce, H., W. W. Thatcher, D. E. Buffington, C. J. Wilcox, and H. H. Van Horn. 1977. Physiological and production responses of dairy cattle to a shade structure in a subtropical environment. *J. Dairy Sci.* 60:424–430.
- Roman-Ponce, H., W. W. Thatcher, D. Caton, D. H. Barron, and C. J. Wilcox. 1978. Thermal stress effects on uterine blood flow in dairy cows. *J. Anim. Sci.* 46:175–180.
- Roman-Ponce, H., W. W. Thatcher, and C. J. Wilcox. 1981. Hormonal interrelationships and physiological responses of lactating dairy cows to a shade management system in a subtropical environment. *Theriogenology* 16:139–154.
- Rosenberg, M., Z. Herz, M. Davidson, and Y. Folman. 1977. Seasonal variations in post-partum plasma progesterone levels and conception in primiparous and multiparous dairy cows. *J. Reprod. Fertil.* 51:563–568.
- Rosenberg, M., Y. Folman, Z. Herz, I. Flamenbaum, A. Berman, and M. Kaim. 1982. Effect of climatic conditions on peripheral concentrations of LH, progesterone and oestradiol-17β in high milk-yielding cows. *J. Reprod. Fertil.* 66:139–146.
- Roush, W. 1994. Population—the view from Cairo. *Science* 265:1164–1167.
- Rutledge, J. J. 1997. Cattle breeding systems enabled by in vitro embryo production. *Embryo Transfer Newsletter* 15(1):14–18.
- Ryan, D. P., J. F. Prichard, E. Kopel, and R. A. Godke. 1993. Comparing early embryo mortality in dairy cows during hot and cool seasons of the year. *Theriogenology* 39:719–737.
- Sabbah, M., C. Radanyi, G. Redeuilh, and E. E. Baulieu. 1996. The 90 kDa heat-shock protein (hsp90) modulates the binding of the oestrogen receptor to its cognate DNA. *Biochem J.* 314:205–213.
- Seath, D. M., and C. H. Staples. 1941. Some factors influencing the reproductive efficiency of Louisiana herds. *J. Dairy Sci.* 24:510 (Abstr.).
- Spain, J. N., and D. E. Spiers. 1997. Effect of niacin supplementation on milk production and thermoregulatory responses of dairy cows. *J. Dairy Sci.* 80 (Suppl. 1):153 (Abstr.).
- Staples, C. R., H. H. Head, and D. E. Darden. 1988. Short-term administration of bovine somatotropin to lactating dairy cows in a subtropical environment. *J. Dairy Sci.* 71:3274–3282.
- Stott, G. H., and R. J. Williams. 1962. Causes of low breeding efficiency in dairy cattle associated with seasonal high temperatures. *J. Dairy Sci.* 45:1369–1375.
- Stott, G. H., F. Wiersma, and J. M. Woods. 1972. Reproductive health program for cattle subjected to high environmental temperatures. *J. Am. Vet. Med. Assn.* 161:1369–1375.
- Sugiyama, S. 1999. Development of a model to study the direct effects of hyperthermia on bovine ovum and embryo development. Ph.D. thesis, University of Queensland, Brisbane.

- Thatcher, W. W., F. C. Gwazdauskas, C. J. Wilcox, T. Toms, H. H. Head, D. E. Buffington, and W. B. Frederksson. 1974. Milking performance and reproductive efficiency of dairy cows in an environmentally controlled structure. *J. Dairy Sci.* 57:304–307.
- Thatcher, W. W., and R. J. Collier. 1986. Effects of climate on bovine reproduction. In: D. A. Morrow (Ed.) *Current Therapy in Theriogenology* 2. pp 301–309. W. B. Saunders, Philadelphia.
- Tompkins, E. C., C. J. Heidenreich, and M. Stob. 1967. Effect of post-breeding thermal stress on embryonic mortality in swine. *J. Anim. Sci.* 26:377–380.
- Trout, J. P., L. R. McDowell, and P. J. Hansen. 1998. Characteristics of the estrous cycle and antioxidant status of lactating Holstein cows exposed to heat stress. *J. Dairy Sci.* 81:1244–1250.
- Turner, H. G. 1982. Genetic variation of rectal temperature in cows and its relationship to fertility. *Anim. Prod.* 35:401–412.
- Ulberg, L. C., and P. J. Burfening. 1967. Embryo death resulting from adverse environment on spermatozoa or ova. *J. Anim. Sci.* 26:571–577.
- Van Amburgh, M. E., D. M. Galton, D. E. Bauman, and R. W. Everett. 1997. Management and economics of extended calving intervals with use of BST. *Livestock Prod. Sci.* 50:15–28.
- Walker, W. L., R. L. Nebel, and M. L. McGilliard. 1996. Time of ovulation relative to mounting activity in dairy cattle. *J. Dairy Sci.* 79:1555–1561.
- West, J. W., B. G. Mullinix, and T. G. Sandifer. 1991. Effects of physiologic responses of lactating Holstein and Jersey cows during hot, humid weather. *J. Dairy Sci.* 74:840–851.
- Wilson, S. J., C. J. Kirby, A. T. Koenigsfield, D. H. Keisler, and M. C. Lucy. 1998a. Effects of controlled heat stress on ovarian function of dairy cattle. 2. Heifers. *J. Dairy Sci.* 81:2132–2138.
- Wilson, S. J., R. S. Marion, J. N. Spain, D. E. Spiers, D. H. Keisler, and M. C. Lucy. 1998b. Effects of controlled heat stress on ovarian function of dairy cattle. 1. Cows. *J. Dairy Sci.* 81:2139–2144.
- Wise, M. E., D. V. Armstrong, J. T. Huber, R. Hunter, and F. Wiersma. 1988a. Hormonal alterations in the lactating dairy cow in response to thermal stress. *J. Dairy Sci.* 71:2480–2485.
- Wise, M. E., R. E. Rodriguez, D. V. Armstrong, J. T. Huber, F. Wiersma, and R. Hunter. 1988b. Fertility and hormonal responses to temporary relief of heat stress in lactating dairy cows. *Theriogenology* 29:1027–1035.
- Wolfenson, D., F. F. Bartol, L. Badinga, C. M. Barros, D. N. Marple, K. Cummings, D. Wolfe, M. C. Lucy, T. E. Spencer, and W. W. Thatcher. 1993. Secretion of PGF₂ α and oxytocin during hyperthermia in cyclic and pregnant heifers. *Theriogenology* 39:1129–1141.
- Wolfenson, D., and O. Blum. 1988. Embryonic development, conception rate, ovarian function and structure in pregnant rabbits heat-stressed before or during implantation. *Anim. Reprod. Sci.* 17:259–270.
- Wolfenson, D., I. Flamenbaum, and A. Berman. 1988. Hyperthermia and body energy store effects on estrous behavior, conception rate, and corpus luteum function in dairy cows. *J. Dairy Sci.* 71:3497–3504.
- Wolfenson, D., W. W. Thatcher, L. Badinga, J. D. Savio, R. Meidan, B. J. Lew, R. Braw-Tal, and A. Berman. 1995. Effect of heat stress on follicular development during the estrous cycle in lactating dairy cattle. *Biol. Reprod.* 52:1106–1113.
- Wolfenson, D., B. J. Lew, W. W. Thatcher, Y. Graber, and R. Meidan. 1997. Seasonal and acute heat stress effects on steroid production by dominant follicles in cows. *Anim. Reprod. Sci.* 47:9–19.
- Wolffe, A. P., A. J. Perlman, and J. R. Tata. 1984. Transient paralysis by heat shock of hormonal regulation of gene expression. *EMBO J.* 3:2763–2770.
- Younas, M., J. W. Fuquay, A. E. Smith, and A. B. Moore. 1993. Estrous and endocrine responses of lactating Holsteins to forced ventilation during summer. *J. Dairy Sci.* 76:430–436.
- Yousef, M. K. 1984. Stress physiology: definition and terminology. In: Yousef, M.K. (Ed.) *Stress Physiology in Livestock*. pp 3–7. CRC Press, Boca Raton, FL.
- Yu, P., J. T. Huber, C. B. Theurer, K. H. Chen, L. G. Nussio, and Z. Wu. 1997. Effect of steam-flaked or steam-rolled corn with or without *Aspergillus oryzae* in the diet on performance of dairy cows fed during hot weather. *J. Dairy Sci.* 80:3293–3297.